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BIOPHYSICAL GENETIC STUDIES USING RADIOACTIVELY TAGGED PROTEINS

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In recent years a number of biological systems have been investigated in which one protein has been altered as a result of mutation. Isolation of the proteins in question has shown a close similarity of their structure with, however, some well-defined differences in behavior. Recently, two such systems have been characterized in some detail. These are: the human hemoglobins A and S, studied by SINGER AND ITANO[1, 2] and β-lactoglobulins A and B, studied by the present authors[3-5]. The latter are two forms of the principal component of milk whey. The purpose of this paper is to compare and contrast the results on hemoglobin (Hb) with those on β-lactoglobulin (Lb).

The various species of both of these systems have been found to dissociate on acidification, while subsequent neutralization reverses this process. Thus, both Lb-A and B, which have a molecular weight of 36,000 at the isoelectric point (pH 5.2), dissociate into half-units at pH's below 3.5[6]. In each of these two cases it is known that a structural difference exists as a result of the mutation. Thus, in sickle-cell hemoglobin (HbS) a valine residue has replaced a glutamic acid present in normal hemoglobin (HbA)[7]. In the case of such dissociable proteins the structural difference may result in various possible types of sub-unit pairs: the structural difference may be localized in one of the sub-units, making these different in each case; the same structural difference may be present in both sub-units, with the result that each molecule dissociates into two identical sub-units. Furthermore, the structural difference may be located either in the area of contact between the sub-units or in a different part of the molecule.

In order to elucidate these questions, hybridization experiments may be carried out. In these experiments, a mixture of the genetic species is brought to conditions of dissociation; then, reassociation is brought about by a reversal of conditions. The resulting protein mixture is examined then for the formation of hybrid molecules formed by the cross-reassociation of the various sub-units. Hybridization, if it occurs, will follow one of two patterns:

Pattern I: if the molecular sub-units are identical:

$$2\,AA + 2\,BB \xrightarrow{\text{acid}} 4\,A + 4\,B \xrightarrow{\text{neutralization}} AA + 2\,AB + BB$$

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Pattern II: if the molecular sub-units are not identical, the entire difference being localized in one sub-unit, the other (N) being common to both species:

$$\begin{matrix} AN \\ + \\ BN \end{matrix} \xrightarrow{\text{dissoc.}} \begin{matrix} A + N \\ B + N \\ \text{(exchange)} \end{matrix} \xrightarrow{\text{reassoc.}} \begin{matrix} AN \\ + \\ BN \end{matrix}$$

In the first case, the sub-unit exchange results in the formation of a new molecular species (AB). In the second case, exchange results in the reformation of species identical with the original ones.

If a difference in electric charge exists between species A and B, then in pattern I the new form AB will have a charge intermediate between the two and in electrophoresis it should migrate with a mobility intermediate between the AA and BB, forming a new third peak in the pattern. In both Hb and Lb a charge difference exists between the two forms. Thus, Lb-A has two more carboxyls than Lb-B per undissociated molecule[8]. Electrophoretic measurements carried out after acidification and neutralization of mixtures revealed no new peak either in Hb[1] or in Lb[5]. Therefore, Pattern I can be eliminated in both cases, and these two protein systems are identical in that they form no mixed hybrid. The question remains, then, does hybridization occur according to Pattern II or is there no hybridization at all?

In order to answer this question, the method of radioactively tagged proteins was employed. In such experiments, protein A is labeled with, for example, ^{14}C, while protein B is left unlabeled. A mixture of labeled A and unlabeled B is acidified and then neutralized. The A and B of the thus treated mixture are then separated and the radioactivity is counted in each. Now, if exchange of groups N had taken place (Pattern II), then, after the dissociation–recombination cycle, 25% of the total radioactivity should have passed from A to B, without any other changes in the properties of the two proteins:

$$\begin{matrix} 2\,A^*N^* \\ + \\ 2\,BN \end{matrix} \xrightarrow{\text{acid}} \begin{matrix} 2\,A^* + 2\,N^* \\ 2\,B + 2\,N \\ \text{(exchange)} \end{matrix} \xrightarrow{\text{neutral}} \begin{matrix} A^*N^* + A^*N \\ BN + BN^* \end{matrix}$$

If no exchange takes place, the entire original radioactivity should be recovered in protein A.

Such experiments were carried out in Lb[5] as well as in Hb[2]. The acidified, then neutralized protein mixture was resolved into its components electrophoretically and the components were sampled out of the Tiselius cell (in Lb satisfactory resolution of the electrophoretic components is obtained at pH 5.3[9]). The amount of radioactivity in each was then counted. It was found that the radioactive content of Lb-A had not changed, while Lb-B was found to have a very low count which did not differ from controls in which mixtures which had not been acidified were resolved electrophoretically. Thus, the radioactivity found in Lb-B can be ascribed to a small amount of contamination with Lb-A. This is in contrast to the findings of SINGER AND ITANO[2] who reported that in the case of Hb, the radioactivity of HbA had decreased to about 75% of the original, while that of HbS had acquired about 25% of the total count.

These two systems then differ in their patterns of dissociation and reassociation.

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This must reflect differences in their gross structures. In the hemoglobins, sub-unit exchange occurs as depicted in Pattern II, while in the β-lactoglobulins no such exchange takes place. The structural differences between these two protein systems are: in Hb each molecule is formed of two non-identical sub-units, the difference between types A and S being localized in one of these sub-units; in the Lb the structural difference is distributed between the two sub-units. If this difference reflects a single mutation of the gene, then it must be identical in the two sub-units, and furthermore the sub-units of each species of Lb must be identical. The inability of the β-lactoglobulins to form hybrids also proves that the structural difference between Lb-A and Lb-B is localized in that region of the molecule where the two sub-units are linked together; had no difference been present in that area, hybrid molecules of type AB (pattern I) would have been possible, the bond holding the sub-units together being identical in the two species.

Comparison of the results obtained with β-lactoglobulin with those obtained with hemoglobin points at two types of structural patterns that may exist in dissociable genetically related proteins; it further demonstrates the usefulness of radioactively tagged proteins in studies in biophysical genetics.

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